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Evaluation of GeneXpert MTB/RIF Assay for Rapid Diagnosis of Tuberculosis and Detection of Rifampicin Resistance in Extra Pulmonary Specimens

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ABSTRACT

Introduction: Extra pulmonary tuberculosis (EPTB) accounts for 15%-20% of all tuberculosis (TB) cases. Diagnosis of EPTB is difficult due to its pauci-bacillary nature. The slow growth rate of mycobacterium makes isolation and identification by culture delayed. The Xpert MTB/RIF assay detects both TB and resistance to rifampicin in less than two hours. Hence, we compare Gene Xpert with TB culture to diagnose EPTB and determine rifampicin resistance**.**
Methods: The study was conducted between Nov 2016 to Oct 2017 and all EPTB specimens were collected. The collected specimens were processed for acid fast stain, MTB/RIF assay and liquid culture by MGIT (Mycobacterial Growth Indicator Tube).
Results: A total of 340 EPTB specimens were evaluated. Culture positivity rate was 22.4%. Gene Xpert was positive in 21.7% cases. The sensitivity of Xpert was 68% and specificity was 92% with culture as gold standard. The sensitivity of Gene Xpert in various samples ranged from 93% to 12% being highest in pus sample (93%) and lowest in pleural fluid (13%) whereas the specificity reversed being 100% in pleural fluid and 79% in pus. MTB detected 22 more cases than culture hence Xpert identified 29.7% more cases than culture. We also observed that Xpert missed 9% culture positive cases.
The culture confirmed rifampicin resistant was observed in 12 cases while Gene Xpert identified in 8 among them however in 3 cases Xpert was rifampicin sensitive.
Conclusion: Our study confirms that Xpert MTB/ RIF assay is an important advancement in diagnosing EPTB. Although it cannot replace the conventional techniques but can contribute significantly in an early diagnosis and management of EPTB . Hence a combined approach including acid fast stain, culture identification and sensitivity and Gene Xpert as well as appropriate sample should be sent for an early and reliable diagnosis of EPTB.

Keywords: Extra pulmonary tuberculosis, *Mycobacterium tuberculosis*, Gene Xpert MTB/RIF assay, rifampicin resistance, MGIT (mycobacterial growth indicator tube)

INTRODUCTION

Tuberculosis (TB) remains a major global public health problem with one third of the world's population being infected with *Mycobacterium tuberculosis*. [1] Pulmonary tuberculosis is the most common form of human tuberculosis. However, in recent years increase in the proportion of cases diagnosed as extra pulmonary tuberculosis (EPTB) has been observed. [2] In

developing countries like India, the percentage of EPTB cases is between 15% - 20%, which has increased to more than 50% among the HIV co-infected patients.[3] The synergism between drug resistance TB and HIV has further complicated the scenario. [1,4] The diagnosis of EPTB poses a challenge for clinicians as it requires high clinical suspicion. Since EPTB can affect virtually all organs, it has varied clinical manifestations. The pauci-bacillary nature along with need for invasive procedures to obtain appropriate sample from relatively inaccessible sites, make EPTB diagnosis difficult.[5] The definitive diagnosis of EPTB involves demonstration of *M. tuberculosis* by microbiological, histo-cyto pathological methods.[6] Isolation of *M. tuberculosis* from clinical samples by culture is the "gold standard, however due to slow growth rate of mycobacterium, isolation and identification by culture takes several weeks or longer. In 2013, the World Health Organization (WHO) updated its policy and endorsed Xpert MTB/RIF assay for diagnosis of EPTB. [7] The Xpert MTB/RIF assay remains the fully automated cartridge-based DNA test that can detect both TB and rifampicin resistance in less than 2 hours. In this study we compare liquid culture and Gene Xpert for identification of *Mycobacterium Tuberculosis* and diagnosis of extra pulmonary tuberculosis in a clinically suspected patient.

MATERIALS AND METHOD

This was a prospective observational study conducted from Nov 2016 to Oct 2017 in a tertiary care hospital in North India. This study was reviewed and approved by the Institutional Review Board of the institute (Reference no. MICR-664/2016). Individual informed consent was waived by the ethics committee because the samples collected during routine medical care were included and did not pose any additional risks to the patients. However, patient's information was anonymized prior to the analysis.

A total of 340 extra pulmonary samples were included from patients suspected of having tuberculosis. The samples were processed for acid fast stain, culture identification and sensitivity by MGIT and Gene Xpert MTB/RIF assay. The extra pulmonary samples included were lymph node aspirates, pus from various anatomical sites, cerebrospinal fluid (CSF), pleural fluid and other sterile body fluids.

The collected specimens were first processed for Ziehl-Neelsen (ZN) stain. The MTB/RIF assay was performed according to the manufacturer instructions. All the samples were then decontaminated by NALC-NaOH method. After decontamination, the samples were inoculated in MGIT liquid medium for culture. For mycobacterium positive cultures, acid fast stain was performed to rule out any contamination. The mycobacterium tuberculosis complex (MTBC) was differentiated from non-tuberculous mycobacterium by using commercially available MPT64 Ag Rapid assay. For MTB complex positive cultures, drug susceptibility testing (DST) was performed using MGIT 320 (Becton Dickinson, USA).

STATISTICAL ANALYSIS

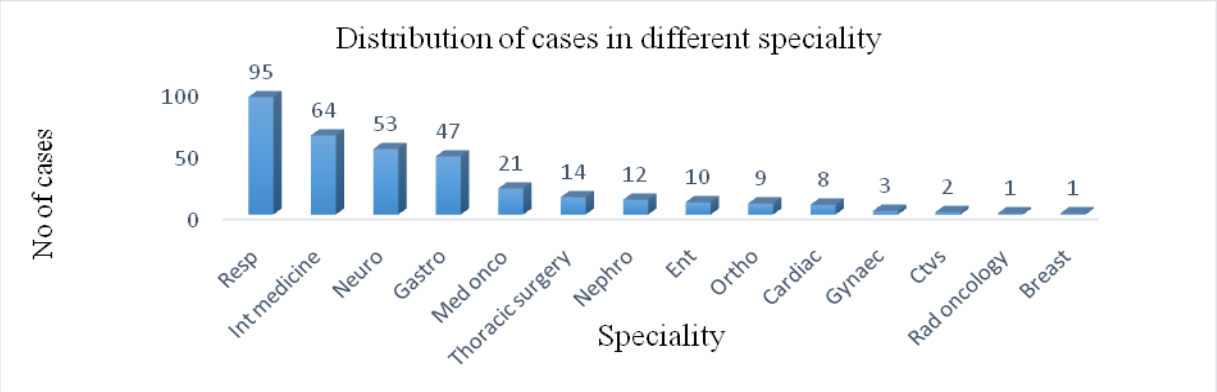
The patient's clinical data was collected from microbiological laboratory database of HIS. The analysis included profiling of patients on different demographic and clinical parameters. Descriptive statistics was analyzed with SPSS software, version 24.0. Categorical variables were expressed as frequencies and percentages. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. Cross tables were generated to illustrate relationship of results by different tests. The comparisons were made using the Chi-square test. P values less than 0.05 was considered as statistically significant.

RESULTS

A total of 340 clinically suspected extra pulmonary tuberculosis patients were included in the study.

The patients included were admitted under different specialties of the hospital predominantly under respiratory medicine 95/340 (28%) and internal medicine department 64/340 (19%). (Figure1)

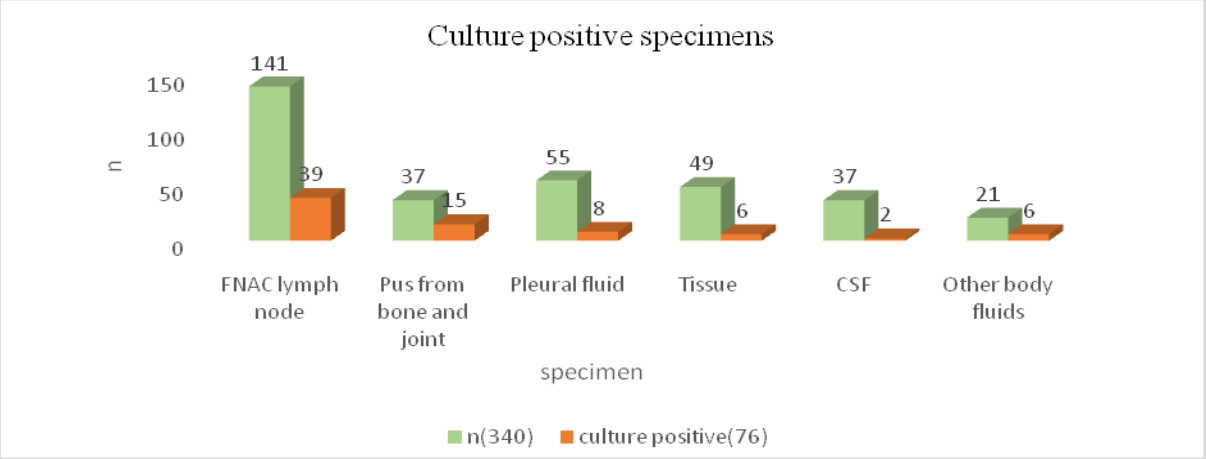
Figure 1: Distribution of cases in different specialties



The most common specimen for extra pulmonary involvement was lymph node 141/340 (42%), followed by pleural fluid 55/340 (16%), tissue (ileocaecal biopsy, vertebral tissue) 49/340 (14%), pus from bone and joints 37/340 (11%), CSF 37/340 (11%), and other body fluids like ascitic fluid, urine, pericardial fluid & pleural fluid 21/340 (6%). (Figure2)

Among 340 specimens, 76 grew *M. tuberculosis* by liquid culture while none of them grew non-tuberculous mycobacterium. The prevalence of EPTB in patients attending our hospital was 22.4% (76/340). Maximum culture positivity was found in pus specimen collected from bone and joint 15/37(41%). (Figure2)

Figure 2: Distribution of culture positive cases in various extra pulmonary specimens



Gene Xpert was positive in 74/340(21.7%) cases. Among 74 Xpert positive cases, culture was positive in 52 cases and grew MTB. The sensitivity of Xpert was 68% (52/76) and specificity of Xpert was 92% (242/264) with culture as gold standard. The positive predictive value was 70% and negative predictive value was 91%. (p value <0.0001). (Table 1)

Table 1: Comparison of Gene Xpert with culture

	Culture positive	Culture negative	Total
Xpert positive	52	22	74
Xpert negative	24	242	266
	76	264	340

The sensitivity of Gene Xpert was variable being highest in pus sample (93%) and lowest in pleural fluid (13%). The specificity of Gene Xpert was 100% in pleural fluid and 79% in pus specimens. (Table 2)

Table 2: Results of Xpert MTB/RIF assay in different extra pulmonary specimens

Specimen n(340)	Xpert positive	Xpert Sensitivity rate	Xpert Specificity rate	Xpert Positive Predictive Value	Xpert Negative Predictive Value
FNAC lymph node (141)	39	79%	92%	79%	92%
Pus(37)	19	93%	79%	74%	95%
Tissue(49)	12	67%	80%	33%	94%
CSF(37)	2	50%	97%	50%	97%
Pleural Fluid(55)	1	13%	100%	100%	87%
Body fluids(21)	1	17%	100%	100%	75%

Among 74 Xpert positive cases, we observed that culture was negative in 22 samples. Hence GeneXpert MTB RIF assay detected 29.7% (22/74) more samples than culture. Among 266 Xpert negative cases, culture was positive in 24 specimens, hence Xpert missed 9% culture positive cases.

In 22 cases where Xpert was positive and culture negative, 16/22(73%) were pus specimens. Also, in 24 samples which were Xpert negative but culture positive most of the samples were fluids (13/24) followed by tissue specimens (10/24).

The positivity rate of culture was 22.4% (76/340) and positivity by Xpert was 21.7% (74/340) but combined positivity rate of both culture and Xpert was 28.8% (98/340)

The acid-fast staining was also done in all patients. The smear was positive in 25 patients and remaining 315 were smear negative. The positivity rate of AFB smear microscopy was 7% in our study. Among 25 smear positive cases, culture was positive in all cases while Xpert was negative in 1 of them. The sensitivity of smear microscopy among the patients with positive cultures were found to be 33% (25/76). The positive predictive value for ZN staining was 100% and negative predictive value was 84%. (Table 3). p value was <0.0001.

Table 3: Comparison of ZN staining with culture

	Culture positive	Culture negative	Total
Smear positive	25	0	25
Smear negative	51	264	315
	76	264	340

Detection of rifampin resistance: Susceptibility testing was performed for all the culture-positive patients and compared it with the performance of Gene Xpert for detecting rifampicin resistance. Among 12 patients with confirmed rifampicin resistant by culture Xpert detected rifampicin resistance in 8 patients only. In remaining 4 patients, rifampicin was sensitive by Xpert in 3 cases and in 1 case, Xpert was negative. The sensitivity of Xpert in detecting rifampicin resistance was 8/12(67%).

DISCUSSION

Tuberculosis remains a key challenge in the face of global public health and inadequate diagnostic techniques have hampered in diagnosing the disease effectively. Diagnosing EPTB is further more difficult as the number of bacilli is less in extra pulmonary samples and obtaining tissues from deep seated organs is difficult. Microscopy, culture and cytology have their own challenges ranging from low sensitivity to long turnaround time. [8] The newer molecular method like Xpert MTB/RIF (Xpert) assay is rapid, detecting tuberculosis and rifampicin resistance within 2 hours.[9] However, Xpert sensitivity varies between different sample types.[9] In this study, we correlate Xpert MTB/RIF assay with liquid culture for mycobacterium tuberculosis to arrive at a definitive diagnosis of extra pulmonary tuberculosis.

The percentage of EPTB cases estimated by the national control program in India for HIV negative adults is between 15% and 20% however our study shows prevalence of 21.9%(73/333) in the patients attending our hospital.[3] The increase in prevalence could be due to availability of more comprehensive tests under one roof.

The remaining 7 patients were diagnosed cases of HIV of which 3 (42.85%) were culture positive for mycobacterium tuberculosis while Xpert detected 4 of them.

The positivity rate of Xpert was 21.7% in our study. We observed that the sensitivity of Xpert was 68% (52/76) and specificity was 92%. The observed sensitivity of Xpert MTB/RIF assay of 68% was consistent with other published studies. They reported sensitivity from 25 to 95% and exceeded 50% in all studies except one study where lower sensitivity was reported in patients with pleural effusion.

Lawn et al in their study reported Xpert MTB/RIF sensitivity 79.0% and specificity 97.3%. [9]

The heterogeneity between different studies could be due to the differences in study design, patient population, and type of EPTB specimen and sample processing. [9]

The sensitivity of Xpert was variable in different samples. The highest sensitivity was found in pus (93%), followed by FNAC lymph node (79%), CSF (50%) and 13% in pleural fluid. The specificity of Gene Xpert was 100% in pleural fluid and 79% in pus specimens.

In a study conducted on EPTB specimens, sensitivity of 60% and 63% and specificity of 100% and 33% for pleural fluid and lymph node tissue samples respectively was observed. [10]

Rufai *et al*. in their study showed that the Xpert MTB/RIF assay has low sensitivity of 14.2% in pleural fluid samples even in culture proven cases. [11]

This ascertains Gene Xpert has high diagnostic potential with good sensitivity for specimens such as pus which is otherwise difficult to diagnose. However

Among 74 Xpert positive cases, we observed that culture was negative in 22 samples. Hence Gene Xpert MTB RIF assay detected 29.7% (22/74) more samples than culture.

This was similar in a study where they reported that Xpert detected 11% more cases than culture. [10]

Among 266 Xpert negative cases, culture was positive in 24 specimens, hence Xpert missed 9% culture positive cases. In samples where Xpert missed MTB detection were mostly fluids where concentration of MTB would be lower than the limit of detection of Gene Xpert MTB RIF assay as a result of Xpert negative and culture positive.

Rifampicin resistance by culture was detected in 12 isolates of which Gene Xpert detected rifampicin resistance in 8 of them. The sensitivity of Xpert in detecting rifampicin resistance was 8/12(67%).

Sharma et al observed sensitivity of 96.3% of Gene Xpert in detecting rifampicin resistance which is higher than the sensitivity found in our study. [12]

Xpert detects rifampicin resistance when it is associated with the mutations in the 81 base pair region of the *rpoB* gene whereas rifampicin resistance can also occur due to the mutation at codon 531 of the rpoB gene or due to S531L mutations as reported by other studies. [13] In our study, 3 rifampicin sensitive cases detected by Xpert that was resistant by culture could be due to the mutations involving the above region.

Xpert assay provides an aid in diagnosing EPTB along with drug sensitivity to rifampicin rapidly when there is high suspicion of EPTB. However in our study the sensitivity of Xpert was extremely heterogeneous, with high sensitivity seen when testing pus, lymph node, tissue samples and cerebrospinal fluid as compared to the pleural fluid and other serous fluids. These findings support the recently released WHO recommendations for the use of Xpert MTB/RIF for tuberculosis diagnosis in CSF and tissue samples.[7]

The limitation of the study was the short study period to accurately predict the prevalence of EPTB. We used only culture as gold standard to analyze the performance analysis of the Gene Xpert MTB/RIF assay however using composite reference standard would provide more accurate analysis of Xpert MTB RIF assay. Also, many a times the quantity of sample obtained from extra pulmonary sites like tissue was a limiting factor in our study.

CONCLUSION

Diagnosis of EPTB poses challenges due to its diversity of symptoms as it can affect virtually all organs and has wide variety of clinical manifestations. The history and clinical examination of the patient should be correlated with laboratory and radiological findings for accurate diagnosis of EPTB. However, with low level of suspicion among clinicians, difficulty in obtaining samples and choice of appropriate diagnostic test for confirmation adds to the challenges faced. The algorithms and diagnostic tools created for confirmation of pulmonary TB are not always applicable for extra pulmonary tuberculosis. In an endemic region like India, it is likely to be missed or under diagnosed. Our study confirms that the Xpert MTB/ RIF assay is an important advancement in the diagnosis of EPTB. Hence combining Gene Xpert MTB/RIF assay on extra pulmonary samples along with other diagnostic techniques can assist in rapid diagnosis of EPTB and have significant impact on the clinical management of disease.

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Conflict Of Interest Statement:

Authors declare: No conflicts of interest

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Ethical Approval: Approved

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